

Microbial Insights into Asthmatic Immunopathology A Forward-Looking Synthesis and Commentary

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Abstract

Asthma is an aberrant inflammatory condition of the airways affecting approximately 1 in 10 children in affluent countries. An increasing body of evidence suggests that microbial exposures during a “critical window” of development in early life play a central role in determining future asthma susceptibility. However, like the disease itself, considerable heterogeneity exists among studies in which researchers have investigated the associations between particular microbial taxa and asthma immunology. As our understanding of asthmatic pathology evolves to enable clearer

definition of asthma endotypes, it will be important to consider the impact of various environmental factors on each endotype. Given the strong evidence in support of the hypothesis that early-life microbial exposures predict later disease states such as asthma, consideration of these endotypes when establishing experimental outcomes in epidemiological studies could allow for increased precision when determining exposure–outcome associations and engaging in more focused follow-up mechanistic investigations.

Keywords: asthma; endotypes; microbiota; immune regulation; perinatal window

(Received in original form July 10, 2017; accepted in final form September 14, 2017)

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R.C.T.B. is supported by a Ferring Studentship Grant Award, a Vanier Canada Graduate Scholarship, a University of British Columbia (UBC) Four Year Doctoral Fellowship, and a Vancouver Coastal Health-Canadian Institutes of Health Research (CIHR)-UBC MD/PhD Studentship Award. B.B.F. is a UBC Peter Wall Distinguished Professor and Canadian Institute for Advanced Research (CIFAR) Senior Fellow. The Finlay Laboratory is supported by operating grants from the CIHR; the Allergy, Genes and Environment Network (AllerGen); and CIFAR-Humans and the Microbiome (HMB). The views expressed in this article do not communicate an official position of the University of British Columbia, CIHR, AllerGen, or CIFAR.

Author Contributions: R.C.T.B. and C.P. drafted the manuscript; B.B.F. guided the development of the manuscript outline; and all authors critically reviewed and revised the content and structure of the document.

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Ann Am Thorac Soc Vol 14, Supplement 5, pp S316–S325, Nov 2017

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DOI: 10.1513/AnnalsATS.201707-534AW

Internet address: www.atsjournals.org

Although the remarkable physical and cognitive changes of perinatal life are often the primary focus of parents' attention, an intricate network of microscopic processes simultaneously works unseen to guide infant immune development that is vital to establishing health later in life (1–3). Successional colonization of the intestinal tract by certain communities of commensal and symbiotic microorganisms, known as the “gut microbiota,” is increasingly recognized as one such process (4–6). Proposed in the context of a rapidly

increasing prevalence of inflammatory allergic (7) and autoimmune (8) conditions observed primarily in industrialized countries (9, 10), the “microflora hypothesis” suggests a link between dysfunctional microbiota colonization and host immune regulation (11–13). Asthma rates in particular have climbed epidemically, with the disease now affecting over 300 million people worldwide and representing the most frequent chronic condition in the pediatric population (14). The early age of onset, combined with the

current inability to cure or prevent asthma, results in substantial financial costs to health care systems (15, 16) and poses a significant physical, psychological, and financial burden compromising the quality of life of those affected throughout their lifespans.

Given that genetic factors are unlikely to explain the rapid pace at which asthma prevalence has recently increased in Westernized countries, researchers have focused primarily on studying potential environmental factors associated with Westernization that may contribute to

disease development. Among these, delivery by cesarean section (17, 18), lack of dogs in the home (19, 20), antibiotic use (21, 22), reduced farm exposure (20, 23–25), having fewer siblings (26, 27), and a low-fiber diet (28, 29) have all been associated with the development of inflammatory conditions such as asthma. Furthermore, all of these factors have the common feature of being able to disrupt the gut microbiota (30–34).

Notably, an imbalance or disruption of the gut microbiota (dysbiosis) (35–37) and early-life factors capable of altering the microbiota, such as antibiotic use (38, 39), have also been implicated in the development of childhood obesity, another condition steeply on the rise in Western populations (see Koleva and colleagues [40] for a detailed review). Asthma and obesity often co-occur (41, 42), and obese patients with asthma frequently have more severe (43) and/or treatment-resistant (44) forms of the disease. Importantly, obesity differentially relates to asthma severity depending on whether asthma begins early or late in life, with early-onset forms of the disease possibly being perpetuated or exacerbated by obesity (45). In a cross-sectional study from the National Institutes of Health Severe Asthma Research Program, Holguin and colleagues (45) found that, compared with obese individuals with late-onset asthma, obese individuals with early-onset asthma showed increased levels of airway obstruction and bronchial hyperresponsiveness and had more asthma-related health care use. Although the mechanisms linking asthma and obesity remain poorly understood, their mutual responsiveness to dysbiosis suggests that interventions targeting the gut microbiota may represent an important, unexploited means by which to concurrently reduce the incidence of both conditions. Therefore, investigations into interactions between gut microbiota and the immune system will likely provide insights into a common etiology for certain forms of these diseases (46).

The effect of obesity on asthma and the microbiota was recently reviewed by Shore and Cho (47) and Cho and Shore (46). The focus of our present review is therefore to briefly summarize the immunological drivers of asthma pathology and comment on how the complexities of this disease pose a challenge to the comparison of findings derived from various microbiome–asthma association studies. This is followed by a synthesis of what is currently known about the influences of the microbiota on asthma-

related immunopathology, with a focus on early life and severe forms of this disease.

A Window Early in Life Influences Asthma Susceptibility

The exact mechanisms mediating the asthma-associated immunomodulatory effects of the microbiota on the host are only just beginning to be elucidated, but there is increasing evidence to suggest that exposure to a particular community of microbiota is especially important early in life (summarized in Figure 1). During a child's first years of life, geographical location, viral infections, and antibiotic exposure are all significantly correlated with the onset of atopy and asthma (48–50). Moreover, a reduction in the relative abundance of bacteria from the genera *Faecalibacterium*, *Lachnospira*, *Veillonella*, and *Rothia* is observed in the stools of infants at high risk of asthma uniquely during the first 100 days of life; this state of dysbiosis does not persist past this critical window of development (51). Furthermore, recent evidence suggests that the perinatal window of immune system priming by the microbiota may even begin prenatally. Children born from pregnant mothers living on farms prior to delivery have a lower risk of asthma, and increased *in utero* levels of the microbe-derived metabolite acetate are linked to reduced susceptibility to this disease (52, 53). Collectively, these findings suggest that the asthma-protective influence of the microbiota occurs prior to the onset of clinical symptoms. Indeed, much of immune maturation occurs during this peri- and postnatal development period when the microbiota is undergoing the greatest fluctuation.

In agreement with these findings, follow-up studies in which animals were supplemented with bacterial species identified in humans to be protective against asthma development have demonstrated a causal role for the gut microbiota in modulating asthmatic responses (51). Additionally, it has been shown that perinatal, but not adult, antibiotic exposure exacerbates allergic asthma in mice (48, 54). Furthermore, when neonatal mice are exposed to a diverse microbiota, they exhibit reduced circulating IgE levels and airway hyperresponsiveness (AHR) in an experimental model of asthma; however, the same rescuing of symptoms is not observed if mice are colonized later in

life (55). Thus, establishing the mechanisms by which the microbiota governs early-life immune education may have great implications for preventing both asthma and other allergic diseases that plague people in developed countries.

Asthma as a Heterogeneous Disease

Although patients with asthma may present clinically with similar symptoms characterized by wheezing, chest tightness, and shortness of breath, the immunology of asthma is complex and highly heterogeneous at the cellular level (56–59). The existence of defined asthma endotypes, or disease subtypes that differ in their underlying pathophysiological mechanisms, may in part explain discrepancies in treatment responses among patients and inconsistent findings among studies of the causes of asthma (57, 58). This is especially true in the case of the microbiota. Although asthma endotype definitions are constantly evolving and it is unlikely that endotypes are mutually exclusive, understanding and clearly defining the cellular mechanisms mediating asthma immunopathology will allow researchers to link these mechanisms to known microbiota-mediated immunomodulatory effects.

The historical paradigm of asthma as either an early-onset, atopy-associated (extrinsic) or adult-onset, nonatopic (intrinsic) disease (60, 61) is overly simplistic and has largely been abandoned. However, this classification system still provides a useful framework from which disease endotypes can begin to be conceptualized (62). A distinction between early- and late-onset forms of asthma is still supported by current literature (63, 64), but more specific endotypes likely exist within these groups (62). Childhood atopic asthma is the most commonly recognized form of the disease and is classically associated with a family or personal history of allergic disease and the development of an adaptive immunity-driven, type 2 allergy-like inflammatory response to inhaled allergens involving T-helper cell type 2 (Th2) cells; IL-4, IL-5, and IL-13 cytokines; eosinophilia; and elevated levels of circulating B-cell-derived IgE antibodies (59, 65, 66).

Conversely, intrinsic asthma is classically viewed as a predominantly

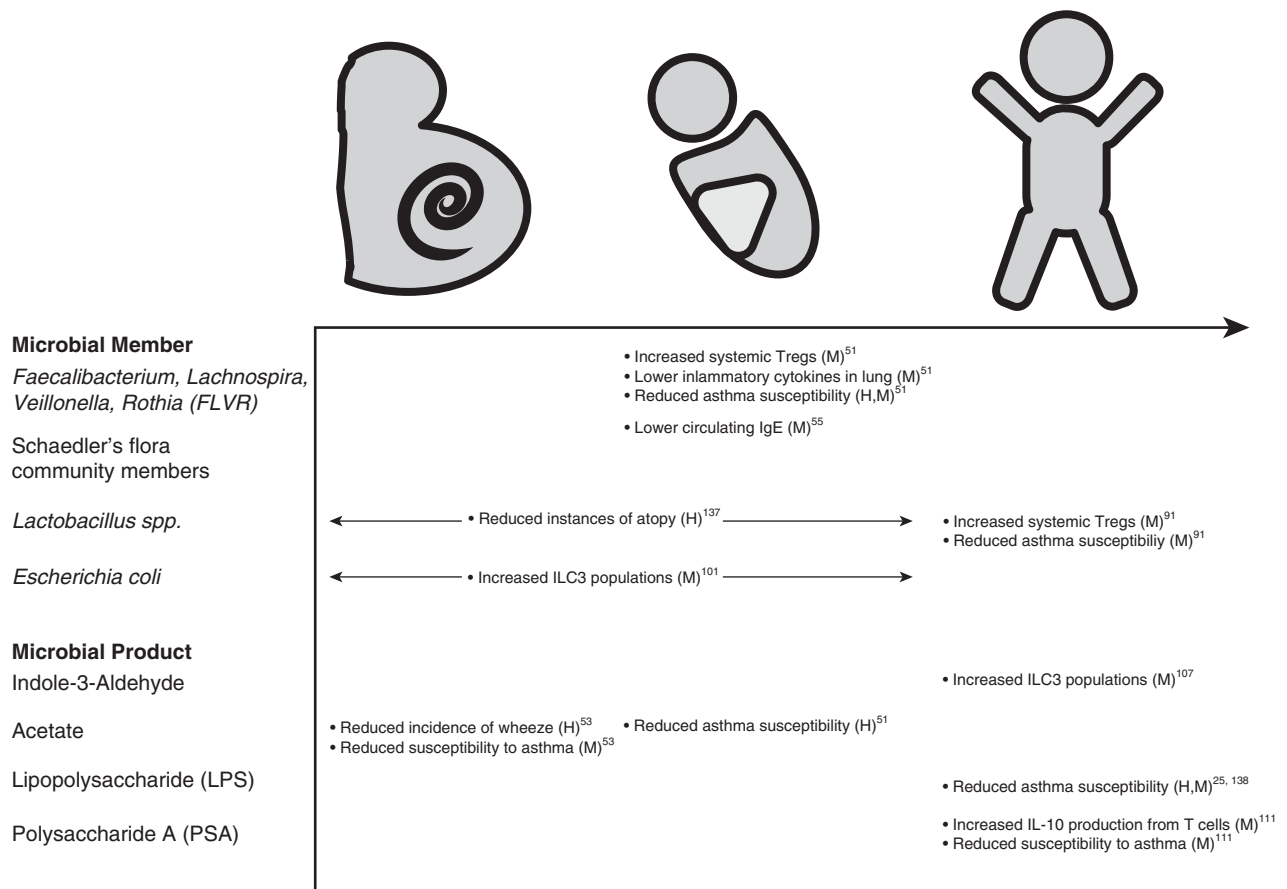


Figure 1. Microbial exposures during specific developmental windows have long-term effects on immunity. Human (H) and mouse (M) studies demonstrating time-specific exposures to microbial members or their products in relationship to immune development and disease susceptibility are illustrated. These exposures include *in utero* (left column), neonatal (center column), and adolescence to adulthood (right column). Superscript numbers refer to References. ILC = innate lymphoid cell; Tregs = T regulatory cells.

nonallergic/non-Th2, innate immunity-driven process (67). Neutrophilic asthma, believed to be driven by Th17 cells and associated signaling pathways, would also fit within the intrinsic asthma category and is typically viewed as the predominant form of late-onset asthma associated with obesity (59, 68). However, more recent evidence suggests that there is significant overlap between the underlying cellular pathophysiology of extrinsic and intrinsic asthma, especially in severe forms of this disease (62).

Severe forms of asthma are associated with both eosinophilia and neutrophilic inflammation (63) and can involve Th17 (69, 70) and Th9 (71) immune mediators in addition to Th2 immunity (65, 72, 73). Moreover, whereas childhood asthma is largely associated with allergic-type Th2 immune responses and eosinophilia, certain forms of severe childhood asthma are

treatment resistant and therefore likely involve additional underlying immunopathology (74–76). Early-onset, obesity-associated asthma appears to be particularly severe (45, 77) and likely involves a mixed Th2/Th17 environment. Indeed, IL-17A-dependent spontaneous AHR has been shown to develop in mice after early-life exposure to a high-fat diet alone (78). Together with evidence that early-onset asthma is typically Th2 predominant, this study provides compelling evidence for the idea that classical early-onset atopic asthma may be further complicated by an innate immunity-driven, obesity-associated asthma in especially severe cases (77).

As more innate and adaptive immune cell types and cytokines have been identified to be important drivers of asthmatic pathology (79), it has become clear that asthma endotype definitions must remain

dynamic and continue to evolve. The incorporation of innate immune cells increasingly recognized for their importance in initiating and sustaining asthmatic inflammation will be especially important in this regard. Such cells include innate lymphoid cells (ILCs) (80, 81), thymic stromal lymphopoietin-secreting epithelial cells (82), and natural killer cells (83, 84).

Asthma Endotypes and the Microbiota: A Potential Explanation for Interstudy Heterogeneity

The idea that different endotypes of asthma result from dysregulation of various immune pathways implies the existence of multiple regulatory mechanisms by which the gut microbiota has the potential to

influence immune development. Studies of associations between the gut microbiota and asthma have been difficult to replicate, which may be due to the use of broadly defined outcomes (most studies simply use “asthma” rather than specific endotypes of asthma as outcomes) and differences in the predominant asthma endotype present in the population studied. If particular groups of microbes differentially modulate alternate immune pathways that ultimately result in asthma, detection of these nuanced influences may be masked in large birth cohort studies using a broad definition of asthma.

Two studies investigating the relationship between the early-life gut microbiota and asthma susceptibility in a Canadian birth cohort highlight the importance of carefully considering asthma outcome definitions when designing and comparing microbiome–asthma association studies. Arrieta and colleagues (51) studied the bacterial content of stool samples from a subset of 3-month old children in the CHILD (Canadian Infant Longitudinal Development) study who developed both atopy and wheeze (a specific phenotype associated with a positive Asthma Predictive Index, in turn associated with later development of early-onset allergic asthma [62]) at 1 year of age. They found that bacteria from the four genera *Faecalibacterium*, *Lachnospira*, *Rothia*, and *Veillonella* were reduced in relative abundance when compared with stool samples from children who remained healthy. However, when stool samples from the same birth cohort were analyzed in relation to a broad diagnosis of asthma that did not necessarily take atopic status into consideration at ages 3–4 years, asthma was associated with a similar but different state of intestinal dysbiosis characterized by reduced levels of *Faecalibacterium* and increased levels of *Clostridium neonatale* (85). Taken together, these studies suggest that the efficacy of putative microbiota-derived therapeutic targets for the prevention/treatment of asthma identified through epidemiological studies will likely be highly specific to the population and/or endotype of asthma to which they are applied.

Immune Cells Required for Asthma Progression Are Regulated by the Microbiota

Despite interstudy heterogeneity among studies of microbiome–asthma associations,

significant advances in the understanding of how microbial exposures influence the onset and progression of this disease have been made. During asthma progression, Th2- and Th17-associated inflammation drives disease within the lungs. However, results of human and mouse studies highlighting the importance of the perinatal period as a critical window of immune development susceptible to microbial influences support the argument that aberrant host–microbe interactions instigate harmful immune development long before clinical symptoms arise.

Central to protection against damaging inflammatory responses are regulatory T cells (Tregs). Tregs are required to dampen both Th2-like and Th17-like immune responses, and their presence in the lungs is reduced in both allergic and obesity-related asthma (86, 87). Germ-free mice have fewer Tregs, and a number of seminal studies have demonstrated the capacity of the microbiota to up-regulate this immune cell in these animals (88–91). The relatively conserved ability of bacterial species across different phyla to influence Treg development highlights their importance for promoting healthy host–microbe interactions and protection against disease. Moreover, researchers in a recent human study observed that, as compared with adult T cells, fetal T cells were more inclined to differentiate into Tregs in response to antigens (92), coinciding with the critical early window of protective education of the immune system by the microbiota.

ILCs represent potent drivers of both Th2 and Th17 immune responses. Through antigen presentation via major histocompatibility complex class II and cytokine expression profiles that mirror T-cell subsets, ILCs constitute a unique bridge between the innate and adaptive branches of the immune system. ILC2s, which express the IL-33 receptor (IL-33R/ST2) and secrete type 2 cytokines in response to IL-33, have recently been implicated in atopic asthma. Genome-wide association studies in humans have identified polymorphisms within the genes encoding for IL-33 and IL-33R that influence the severity of the disease (93, 94). Furthermore, on the basis of a mouse model of IL-33–dependent asthma, researchers recently demonstrated a requirement for ILC2 cells independent of the presence of T- and B-cell populations (95).

Another ILC subset, ILC3s, expresses both IL-17 and IL-22 and has been associated with obesity-related asthma. Circulating IL-17 is detected at higher levels in both patients with severe asthma and obesity, and ILC3-like cells are detected in greater abundance within the lungs of obese patients with severe asthma (78, 96–99). Consistent with these findings, mice fed a high-fat diet have elevated levels of ILC3s within their lungs, and ILC3s alone can induce asthma symptoms in mice lacking T and B cells (78).

Both ILC2 and ILC3 differentiation depend on the microbiota because ILC subsets within germ-free mice have reduced abundance and lower diversity, and they are skewed disproportionately compared with colonized animals (100). Additionally, transient monocolonization of pregnant germ-free dams by a strain of *Escherichia coli* revealed that early microbiota exposure is capable of instigating ILC development (101). Germ-free offspring born to transiently colonized pregnant dams had expanded ILC3 populations and altered expression of ILC2-related genes in this study. Interestingly, exposure of the offspring to the monocolonized mother through either gestation or nursing was capable of altering ILC3 development; however, combined exposure resulted in an additive effect (101).

Mechanisms of Immune Education by the Microbiota

A flexible dialogue has evolved between the host and resident microbes that shapes both immune development and the microbiota architecture from an early age. Seminal studies have begun to reveal a few components of this cross-talk and their impact on health and disease; yet, there are still countless points of interaction that remain to be discovered. Members of the microbiota may influence the host through their expression of surface antigens and/or metabolic output. We focus our attention on those microbial factors that have been demonstrated to promote the development of immune cells implicated in asthma protection or that play a direct role in reducing asthma susceptibility.

Microbe-derived Metabolites

In addition to having direct effects on the immune system, the collective genome of

the gut microbiome confers functional abilities to this community that extend beyond those of the human host. This includes the metabolism of dietary fiber into metabolites such as short-chain fatty acids (SCFAs) as well as bile acid processing and hormone recycling. Notably, redundancy in the functional abilities of different bacteria means that different community structures may retain similar functional capacities.

Recently, a wealth of studies have demonstrated the influence of SCFAs on various immune cells and diseases. As microbe-derived products from soluble fiber, acetate, propionate, and butyrate make up the largest proportion of SCFAs within the gut and are also found in systemic circulation. Exposure to a high-fiber diet has recently been demonstrated to improve asthma symptoms in both clinical and mouse asthma studies, and treatment of pregnant dams with a high-fiber diet or supplementing their drinking water with SCFAs reduces asthma symptoms in their offspring (53, 102, 103). SCFAs protect against asthma and promote Treg development through G protein-coupled receptors 41 and 43 and have been demonstrated to passively diffuse into cells and influence histone acetylation (102, 104, 105). Indeed, the protective effects of SCFAs during perinatal development in mouse studies have been attributed to their ability to influence epigenetic changes in Tregs, suggesting a potential mechanism by which early life exposure could result in life long alterations to T cells (53).

Another microbe-derived metabolite, indole-3-aldehyde, is a downstream product of tryptophan metabolism. Indole-3-aldehyde is produced by multiple members of the microbiota and signals through the host receptor, aryl hydrocarbon receptor, to influence ILC3 populations within the gut (106, 107). Thus, evidence is accumulating to suggest that the combined contribution of dietary intake and the metabolic capacity of the microbiota has the potential to influence immune development and disease progression of multiple asthma endotypes.

Microbe-associated Molecular Patterns

In addition to producing metabolic products that influence host immune pathways, microbes express a variety of surface antigens that are recognized by cells from both the innate and adaptive arms of the immune system. One of the best-studied

microbe-associated molecular patterns is LPS, the major component of the outer membrane of gram-negative bacteria. Enhanced exposure to LPS during early life is negatively associated with asthma sensitization, and polymorphisms within two LPS recognition proteins, CD14 and Toll-like receptor 4, influence susceptibility to both nonatopic and atopic asthma (52, 108, 109). In support of this, a recent study showed that exposing mice to low doses of LPS prior to the induction of asthma led to reduced AHR and a more tolerant immune response (25). However, LPS is a heterologous molecule that varies between species, and recent evidence suggests that the type of LPS exposure during early development might be just as important as the total amount (110).

Another surface antigen, polysaccharide A, is a bacterial capsular component of *Bacteroides fragilis* that has been studied for its ability to promote the development of Tregs within the gut (89). Although originally characterized for its influence in gut-specific inflammation, researchers recently demonstrated that either colonization of *B. fragilis* or treatment with polysaccharide A alone protected animals from experimental asthma as well (111). Although these represent only a few examples, together these studies provide a framework for exploring the wealth of immunomodulatory surface antigens that likely exist within the gut.

The Microbiota and Asthma: An Ever-Evolving Story

The Gut Mycobiome

Given their numerical dominance within the gut microbiota community, bacteria have received the most attention in microbiome research thus far. However, the gut is also home to a collection of symbiotic archaeal, viral, and eukaryotic microbes. Recent attention to the fungal microbiome, known as the “mycobiome,” indicates that fungi in particular are an important part of the gut microbiota susceptible to environmental factors and potentially relevant to linkages found between the microbiome and disease states, including asthma and allergy (112–118).

Several lines of evidence support the role of fungi in immunomodulation. Fungal antigens can themselves act as allergens, and

various fungal cell components can stimulate the immune system through receptors shared with common allergens and bacteria (115, 119–122). Thus, it is possible that fungal activation of these receptors modulates subsequent immune responses to antigens shared with common allergens by directly or indirectly influencing innate and subsequent adaptive immune responses to these antigens. Notably, mannan in the cell wall of the yeast *Saccharomyces cerevisiae* is thought to bind to airway smooth muscle cell mannose receptors known to be involved in the uptake of house dust mite proteins commonly associated with human allergies and asthma. Exposure to this polysaccharide prior to asthma induction reduces airway inflammation, mucous hypersecretion, hyperresponsiveness, and remodeling in a mouse model of asthma (123). These data suggest that whereas certain fungal antigens and species may exacerbate asthma-related immunopathology, others may protect against certain aspects of the pathological changes observed in the lungs of patients with asthma.

A recent study by Fujimura and colleagues suggests that fungal dysbiosis in the gut may contribute to disease states within the lung (118). These authors examined the fungal and bacterial contents of stool samples taken from infants approximately 1 month old and found that those infants at high risk of developing asthma by age 4 years exhibited a distinct state of bacterial and mycological dysbiosis as compared with healthy children (118). Children with the highest risk of developing asthma in this study exhibited lower relative levels of fecal *Malassezia* taxa and higher relative levels of several *Candida* and *Rhodotorula* taxa (118).

Complexities in the directionality of fungal influences on asthma susceptibility are further evidenced by data from the Epidemiology of Home Allergens and Asthma prospective birth cohort study done in Boston, Massachusetts. This study showed that in infants with a parental history of asthma, hay fever, or allergy, the presence of yeasts in house dust reduced the infants' propensity to develop asthma by ages 12–13 years (124). At the same time, these authors also found that exposure to air- and dustborne *Alternaria* and *Cladosporium* organisms, respectively,

trended toward having an asthma priming effect (124).

In addition to house dust, other environmental exposures associated with both disruption of the microbiota and asthma susceptibility may involve the mycobiome. For instance, fungi often flourish after antibiotic exposure, potentially contributing to linkages found between antibiotic use in early life and the later development of asthma and allergies. Indeed, Noverr and colleagues showed that 5 days of antibiotic exposure followed by a single exposure to the yeast *Candida albicans* was sufficient to induce a potent CD4-mediated Th2 allergic response in the lungs of mice after intranasal exposure to spores from the mold *Aspergillus fumigatus*, a common human allergen (125). Fungi and bacteria physically interact in the gut, produce molecules that influence the growth of one another, and manipulate how the host engages with different microbes (115, 126). Therefore, elucidating the mechanisms driving associations found between bacterial and fungal disease-associated states of dysbiosis may represent a rich source of health-promoting or therapeutic targets with the potential to modulate disease outcomes and development (127).

As researchers continue to probe the gut microbiota for insights into human disease states, it will be important to determine how environmental exposures mold the resident microbial (bacterial and mycological) ecosystem within the gut,

whether fungal dysbiosis is a cause or consequence of either bacterial dysbiosis or an unexplored host or environmental factor leading to both asthma and fungal dysbiosis, and the mechanisms mediating potential asthma-related host immune cell-fungal interactions.

The Lung Microbiome

As the field of microbiome research continues to grow, the ongoing development of advanced tools and techniques aimed at studying the microbial communities within and on human hosts has afforded researchers the ability to identify and study unculturable microbes isolated from body sites previously thought to be sterile. The lungs are one such site.

It is now recognized that the lung microbiome differs in composition among subjects with and without asthma (128, 129) and that differences in this community exist even among patients with asthma of varying severities (130). Studies showing an impact of the lung microbiome on asthma-related immunopathology and the mechanisms mediating these impacts are accumulating (131–133). However, despite evidence to suggest a role for the early-life lung microbiome in the development of lung diseases (134), there is a paucity of studies thus far investigating whether dysbiosis specifically in the early-life lung microbiome is present prior to the onset of asthma symptomatology. With the continued advancement of sequencing technologies, consideration of this microbial community,

including its fungal components (135), has the potential to provide further insights into the mechanisms by which early-life microbial exposures modulate asthma susceptibility.

Conclusions

Microbial exposure in early life is now well established as an important factor in driving healthy and pathological immune development. In the context of early-onset asthma, a heterogeneous disease involving multiple cell types, these exposures have been linked to protection against Th2- and Th17-mediated inflammation through interactions with the immune system via microbial structural features and metabolic products. Obesity-associated asthma represents an especially difficult to treat form of asthma involving dysregulation in both of these arms of the immune system, suggesting a possible disease-modifying potential for certain early-life microbial exposures. As precision medicine moves to the forefront of health care initiatives (136), consideration of individual asthma endotypes and population-specific states of microbial dysbiosis in patient-specific asthma treatment and research programs will be critical to the attainment of improved clinical asthma outcomes (72, 77). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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